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Novel pathogenic mutations in minichromosome maintenance complex component 9 (*MCM9*) responsible for premature ovarian insufficiency

Ting Guo, M.D., Ph.D.,[a] Ye Zheng, M.D., Ph.D.,[a,b] Guangyu Li, M.Sc.,[a] Shidou Zhao, M.D., Ph.D.,[a] Jinlong Ma, M.D., Ph.D.,[a] and Yingying Qin, M.D., Ph.D.[a]

[a] Center for Reproductive Medicine, Shandong University, National Research Center for Assisted Reproductive Technology and Reproductive Genetics, and Key Laboratory of Reproductive Endocrinology (Shandong University), Ministry of Education, Jinan; [b] Department of Reproductive Medicine, the Affiliated Hospital of Qingdao University, Qingdao, 266000, Shandong, People's Republic of China

Objective: To investigate whether mutations in the minichromosome maintenance complex component 9 (*MCM9*) gene were present in 192 patients with sporadic premature ovarian insufficiency (POI) of Chinese descent.
Design: Genetic and functional study.
Setting: University-based reproductive medicine center.
Patient(s): A total of 192 patients with sporadic POI and 192 control women with regular menstruation.
Intervention(s): Sanger sequencing performed in 192 sporadic POI patients, and potential pathogenic variants were excluded in matched controls. Functional effects of mutations on MCM9 were explored based on etoposide-induced DNA damage response, and DNA repair capacity was evaluated by histone H2AX phosphorylation level.
Main Outcome Measure(s): Sanger sequencing and functional characteristics.
Result(s): Three novel heterozygous mutations in *MCM9*, c.C1423T (p.L475F), c.T2921C (p.L974S), and c.G3388A (p.A1130T), were identified in three POI patients separately, which were absent in 192 controls. Functional studies showed that the human embryonic kidney 293 (HEK293) cells overexpressing mutant *MCM9* presented with diminished DNA repair capacity compared with wild type.
Conclusion(s): This study identified novel mutations in *MCM9* that are potentially causative for sporadic POI in Chinese women and further highlighted the role of DNA repair capacity in maintenance of ovarian function. (Fertil Steril® 2020;113:845–52. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: DNA repair, MCM9, mutation, POI

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Premature ovarian insufficiency (POI) is defined as loss of ovarian function before 40 years of age, characterized by amenorrhea, infertility, estrogen deprivation, and elevated follicle-stimulating hormone (FSH) serum levels (1). Approximately 1% to 5% of women are affected by POI and are at high risk of osteoporosis, cardiovascular disease, and other long-term health complications due to estrogen deficiency (2).

Premature ovarian insufficiency is a heterogeneous condition both clinically and etiologically. Besides iatrogenic factors such as ovarian surgery, chemotherapy, or radiation therapy, POI may be caused by chromosomal abnormalities, gene mutations, immune disease, or infections, although

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Reprint requests: Yingying Qin, M.D., Ph.D., 44 Wenhua Xi Road, Jinan, Shandong, 250012, People's Republic of China (E-mail: qinyingying1006@163.com).

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in most of the patients causes are still unknown (1). Genetic defect accounts for 20% to 25% of POI cases (3). Then causative genes for POI have been found via sanger sequencing according to the phenotypes of animal models, such as *FIGLA*, *NOBOX*, *NR5A1*, *FSHR*, and *BMP15* (3–8), which participate in folliculogenesis or steroid hormone synthesis and response. Recent approaches using whole-exome sequencing in POI pedigrees have found a few novel causative genes involved in meiosis or DNA damage repair, such as *HFM1*, *STAG3*, *SYCE1*, *MCM9*, *MCM8*, *MSH5*, and *BRCA2* (9–17).

Among the novel genes, minichromosome maintenance complex component 8 (*MCM8*) and 9 (*MCM9*) are DNA helicases participating in DNA replication and homologous recombination (HR), which are crucial for gonadal development and ovarian function (18). *Mcm9* knockout mice have atrophic ovaries completely devoid of follicles (19, 20). Homozygous pathogenic variants in *MCM9* have been found in POI pedigrees (14, 21). Desai et al. (22) found nearly 5% of patients with sporadic POI carried damaging heterozygous mutations of *MCM9*. However, the contribution of *MCM9* for POI in Chinese patients is unclear. Here, we performed Sanger sequencing of *MCM9* in 192 Chinese women with sporadic POI and identified three novel heterozygous mutations. Functional studies found the three mutations impaired DNA repair efficiency of *MCM9*, indicating that haploinsufficiency of *MCM9* contributes to pathogenesis of POI in women of Chinese ethnicity.

MATERIALS AND METHODS

Study Population

A total of 192 patients with sporadic POI and 192 control women were recruited from the Center for Reproductive Medicine at Shandong University. The criteria for sporadic POI included primary or secondary amenorrhea for at least 4 months before 40 years of age, along with at least two instances of serum FSH levels >40 IU/L detected at an interval of 4–6 weeks, 46,XX karyotype, and no family history of POI. Known causes, such as autoimmune diseases, pelvic surgery, and chemo/radiotherapy treatment were excluded.

As controls, we recruited 192 women with regular menstruation and normal levels of FSH (<10 IU/L), who were receiving intracytoplasmic sperm injection (ICSI) treatment owing to male factor infertility. All the control women had normal ovarian responses during ovarian stimulation (Table 1).

Written informed consent was obtained from all participants. This study was approved by the institutional review board of Reproductive Medicine at Shandong University.

Sanger Sequencing

Genomic DNA was extracted from peripheral blood samples with QIAamp DNA minikit (Qiagen) according to the manufacturer's protocol. All exons and exon-intron boundaries of human *MCM9* gene (ENST00000316316.10) was amplified by polymerase chain reaction (PCR). The PCR products were purified, labeled by Bigdye (Applied Biosystems), and sequenced on an ABI 3730-Avant Genetic Analyzer (Applied Biosystems). All the variants were confirmed by three independent PCR runs, sequenced in forward and/or reverse directions. The novel variations were verified in the 192 controls. Amino acid sequences of other species were obtained from the Uniprot database, and the conservation analysis was conducted on the ClustalW2 Web site (www.clustal.org/clustal2).

TABLE 1

Clinical features of patients with sporadic premature ovarian insufficiency and controls.

Characteristics	Sporadic POI	Control	*P* value
No. of patients	192	192	—
Age (y)	28.75 ± 4.22	27.03 ± 2.74	<.001
Age of amenorrhea (y)	23.01 ± 5.19	—	—
FSH (IU/L)	77.07 ± 25.84	6.02 ± 1.08	<.001
Amenorrhea type			
Primary (n)	14	—	—
Secondary (n)	178	—	—

Note: FSH= follicle-stimulating hormone; POI = primary ovarian insufficiency.

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Plasmids Construction and Mutagenesis

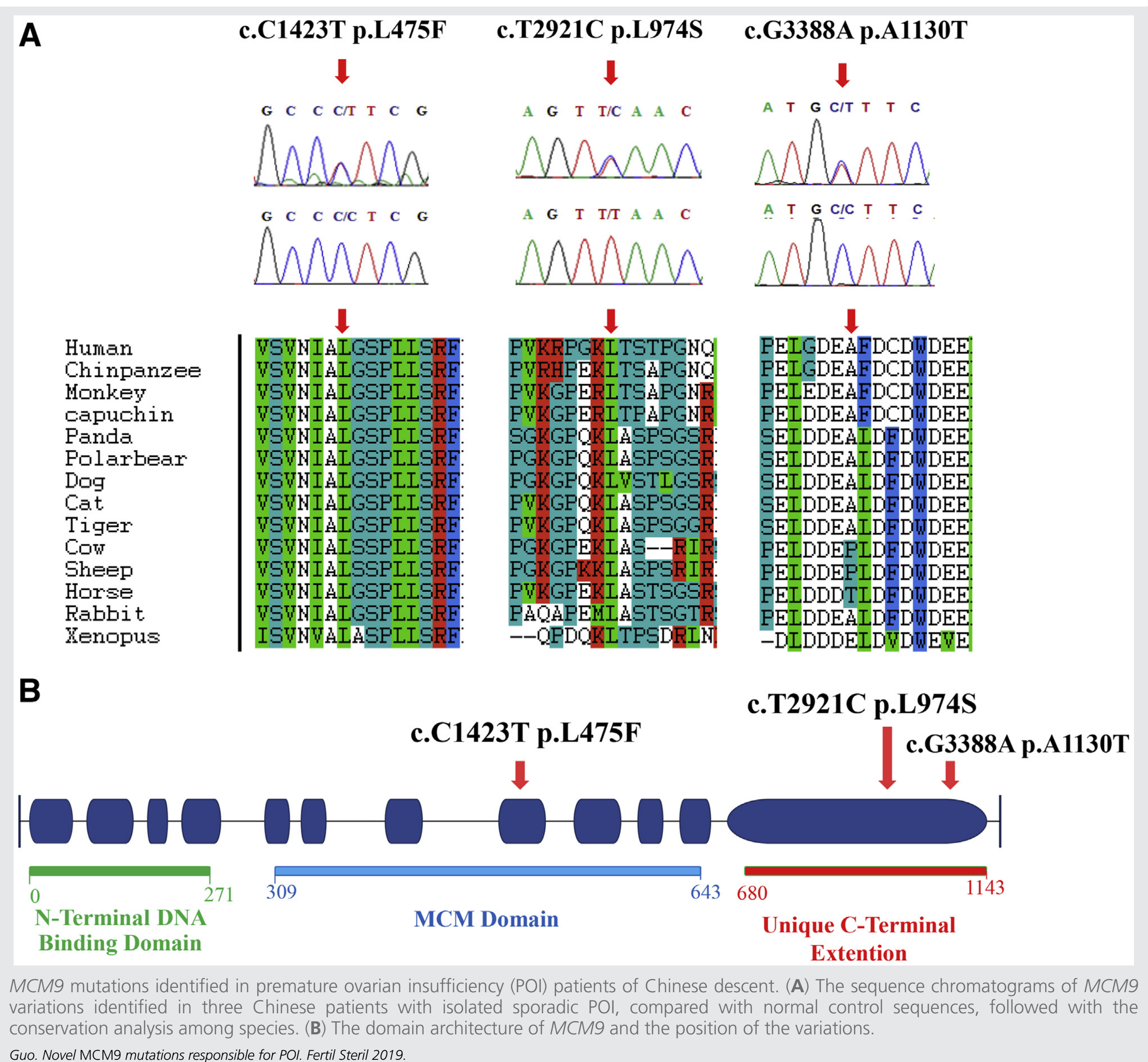
The wild-type plasmid was constructed by inserting human *MCM9* cDNA directly into the pcDNA3.1 vector. The mutant plasmids were generated with wild-type plasmid as the template, using the QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent), and were confirmed by Sanger sequencing.

DNA Repair Assay

Human embryonic kidney 293 (HEK293) cells were cultured in Dulbecco's modified Eagle's medium/high glucose (ThermoFisher) medium supplemented with 10% fetal bovine serum at 37°C with 5% CO_2 for 24 hours. They were then transiently transfected with wild-type or mutant MCM9 plasmids with the use of lipofectamine (Invitrogen). After 24 hours, the cells were incubated in culture medium containing etoposide (ETO, 5 μg/mL) for 2.5 hours at 37°C to induce DNA double-strand breaks (DSBs). Then the culture medium was dropped, and the cells were harvested immediately, or they were cultured with normal medium for an additional 3 or 6 hours at 37°C whereupon the cell were harvested after recovery. Phosphorylation of the Ser-139 residue of histone variant H2AX (γH2AX; Cell Signaling Technology) was tested as a sensitive marker for DSBs by use of Western blot analysis. To further assess the potential dominant negative effect, the mutant plasmids and wild-type plasmids (in a 1:1 ratio, amount equal to isolated wild-type group) were cotransfected into HEK293T cells, followed by the DNA repair assay.

Three independent experiments were conducted. To compare the change in γH2AX level, we quantified the grayscale scores of Western blot bands using ImageJ software (National Institute of Health). The grayscale scores of γH2AX in the cells treated with ETO and recovered at specific time points were divided by those of untreated cells in each group. We compared the relative grayscale score between the wild-type and the mutant groups after 6 hours of recovery.

FIGURE 1



MCM9 mutations identified in premature ovarian insufficiency (POI) patients of Chinese descent. (**A**) The sequence chromatograms of *MCM9* variations identified in three Chinese patients with isolated sporadic POI, compared with normal control sequences, followed with the conservation analysis among species. (**B**) The domain architecture of *MCM9* and the position of the variations.

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GFP-based HR Reporter Assay

The HEK293 cells carrying green fluorescent protein (GFP)-cased HR reporter substrates and I-SceI-vector were generously provided by professor Fengli Wang from Huazhong University of Science and Technology and professor Hailong Wang from Capital Normal University (23). The DSBs could be generated by transfecting I-SceI into the HR reporter cells. If the DSBs had been repaired through HR, GFP would be expressed, and the percentage of GFP-positive cells would reflect the HR efficiency. Therefore, we cotransfected I-SceI and MCM9-pcDNA3.1 plasmids (wild type and/or mutant) into the HR reporter cells.

The cells were collected for flow cytometry analysis after 48 hours of culture. The pcDNA3.1 vector was transfected into the HR reporter cells as negative controls (NC). The percentage of GFP-positive cells was calculated, and the ratio of HR efficiency was obtained by the percentage of GFP-positive cells in the wild-type or mutant groups divided by that of NC group. Three independent experiments were conducted, and at least 40,000 cells were counted at one time.

Statistical Analysis

Software SPSS 20 (IBM) was used for data analysis. The age and serum FSH concentration were checked for normality and described as mean ± standard deviation. The frequency of genotypes was tested by Pearson's chi test or Fisher's exact test. The relative grayscale of the Western blots and HR repair efficiency were tested by independent sample *t*-tests. All the *P* values were two-sided, and $P<.05$ was considered statistically significant.

RESULTS

Three Novel Mutations Identified in POI

Through Sanger sequencing in 192 patients with POI, three novel heterozygous missense variations in *MCM9* (ENST00000316316.10) were identified: c.C1423T (p.L475F), c.T2921C (p.L974S), and c.G3388A (p.A1130T), which were absent in 192 controls. Variants p.L475F and p.L974S were highly conserved across species (Fig. 1). Variant p.L475F in exon 8 was located at the minichromosome maintenance (MCM) domain, which promotes hydrolysis of adenosine triphosphate (ATP), and p.L974S and p.A1130T in exon 12 were located at the C-terminal extension domain. In addition, 12 single nucleotide polymorphisms (SNP) were identified (Supplemental Table 1, available online). Among them, the frequencies of the SNPs rs768968338 (allele frequency: 99.74% vs. 99.994%, $P=.043$) and rs79670608 (allele frequency: 99.74% vs. 98.24, $P=.02$) were statistically significantly different between our POI cohort and the 1000 Genomes Project database (www.internationalgenome.org).

The two patients, who carried p.L475F and p.A1130T, experienced menarche at 16 years old, followed by irregular menses (30 to 180 days per menstrual cycle) that ceased at the ages of 25 and 26, respectively. The carrier of mutation p.L974S experienced menarche at 14 years old, had spontaneous menstruation for 4 years, and underwent menopause at the age of 19. Ultrasound examination of the three patients showed small ovaries with no follicles. None of these patients had a history of pregnancy (Table 2).

Mutant *MCM9*-impaired DSB Repair Capacity

To illustrate the effect of mutants p.L475F, p.L974S, and p.A1130T on DNA repair capacity, we induced DSBs with ETO treatment to evaluate the repair efficiency via the level of γH2AX. In HEK293 cells overexpressing wild-type MCM9, γH2AX increased immediately after ETO treatment and disappeared after recovery for 6 hours. However, the cells overexpressing mutant p.L475F, p.L974S, or p.A1130T showed a higher level of γH2AX after recovery for 3 or 6 hours compared with the wild type.

To examine whether a dominant negative effect of the three mutations existed, we cotransfected the mutant and wild-type plasmids at ratio1:1 into HEK293 cells. The γH2AX level in cotransfected cells after recovery for 3 or 6 hours was lower than that in cells isolated transfected with mutant plasmids but still higher than found in the cells isolated transfected with wild-type *MCM9* after recovery for 6 hours, indicating that haploinsufficiency of mutant *MCM9* would be an possible explanation (Fig. 2A and B). The cells isolated transfected with mutant MCM9 plasmids demonstrated statistically significantly lower HR efficiency than the wild type, whereas the cotransfected cells showed a median HR efficiency between wild-type and mutant cells that was consistent with the results of the DNA repair assays (see Fig. 2C and D).

DISCUSSION

In Chinese patients with POI, our study identified three novel heterozygous missense mutations of *MCM9* that adversely affect DNA repair function, giving more evidence to the pathogenesis of DNA repair defects in the etiology of POI. Primordial follicles are the storage unit of the female germline, and their genetic integrity is essential for maintaining oocyte quantity and quality (24). To protect genome integrity, cells have evolved a network of pathways called DNA damage response, which is responsible for detecting DNA damage, activating checkpoints leading to cell cycle arrest, and coordinating the whole repair process (25). In the germline, DSBs are the most common and severe form of DNA damage, and they are deliberately induced and efficiently repaired through homologous recombination during pachytene of meiosis I (26). Genetic studies of POI have found causative mutations in genes involved in HR, such as *MSH4, MSH5, MCM8, MCM9, HFM1, BRCA2,* and *MEIOB* (10, 13, 14, 16, 27–29), which emphasizes the pivotal role of DNA repair genes in folliculogenesis and maintenance of ovarian function.

The *MCM8–MCM9* complex has been considered to be a participant in DNA replication and resolution of DSBs during HR (30). The two processes are important for germ cell proliferation and meiotic recombination, disturbances of which would lead to insufficient oocyte generation or accelerated oocyte apoptosis (31). Moreover, mice deficient in *Mcm8* and *Mcm9* are infertile and have small gonads due to germ-cell depletion, which mimics the phenotype of POI in humans (20). Whole-exome sequencing has identified homozygous mutations of *MCM8* and *MCM9* in consanguineous pedigrees of POI, suggesting the pathogenetic effect of a dysfunctional *MCM8–MCM9* complex in POI (14, 21). In previous study we found *MCM8* heterozygous mutations in sporadic patients (32). In our present study, we have identified three novel heterozygous missense mutations in *MCM9*. The frequencies of

TABLE 2

Clinical profiles of three women with premature ovarian insufficiency who carry *MCM9* mutations.

Patient	Menarche age (y)	Menopause age (y)	FSH (IU/L)	E_2 (pg/mL)	Stature (cm)	Karyotype	Ovary size (mm) Right	Ovary size (mm) Left	Follicle	*MCM9* variants
POI-1	16	25	138.2	14.4	160	46,XX	15*10	14*8	Absent	c.C1423T (p.L475F)
POI-2	14	18	66.81	<5	153	46,XX	12*5	Invisible	Absent	c.T2921C (p.L974S)
POI-3	16	26	107	<5	160	46,XX	Invisible	Invisible	Absent	c.G3388A (p.A1130T)

Note: E_2 = estradiol; FSH = follicle-stimulating hormone; *MCM9* = minichromosome maintenance complex component 9; POI = premature ovarian insufficiency.

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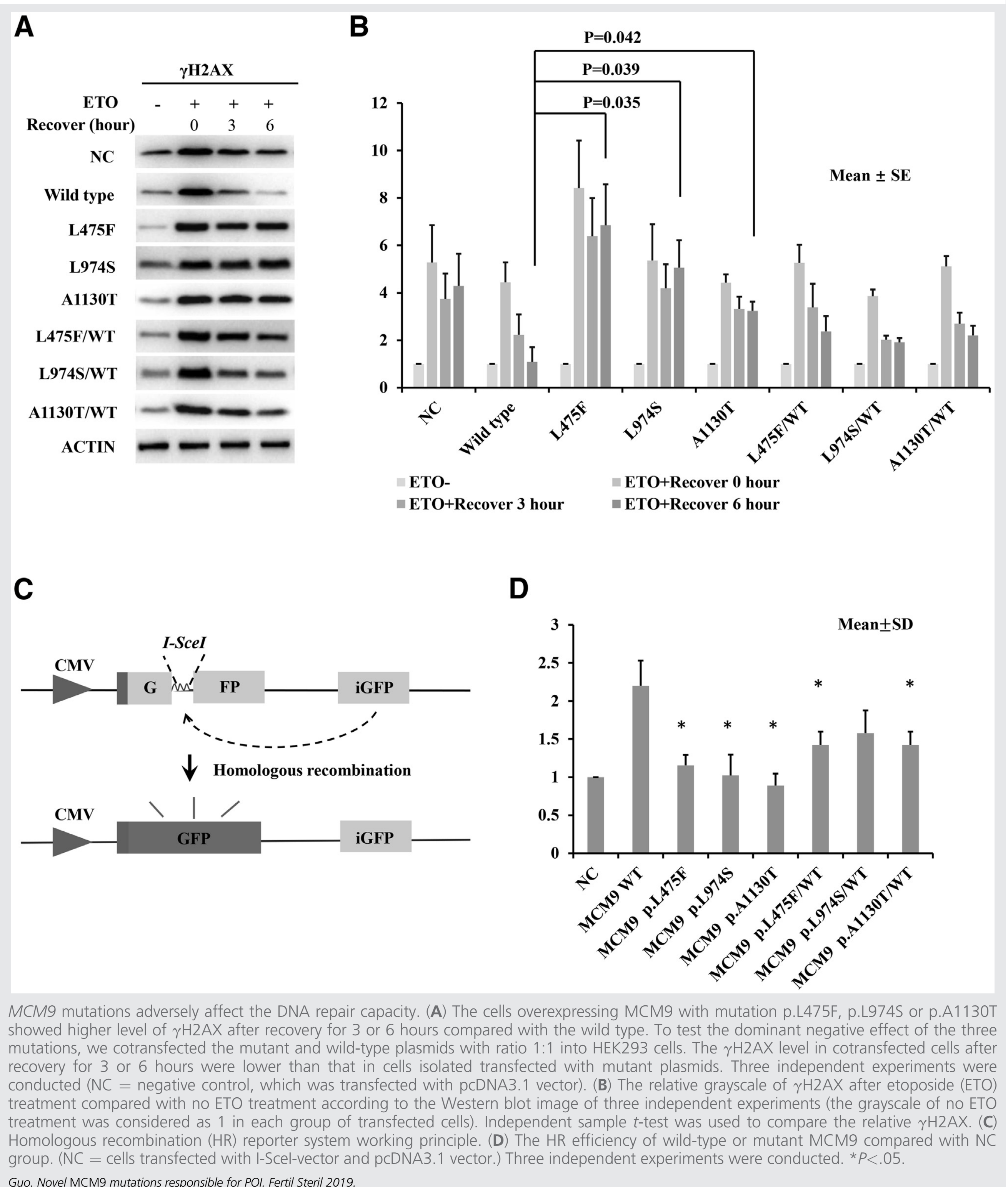
FIGURE 2



MCM9 mutations adversely affect the DNA repair capacity. (**A**) The cells overexpressing MCM9 with mutation p.L475F, p.L974S or p.A1130T showed higher level of γH2AX after recovery for 3 or 6 hours compared with the wild type. To test the dominant negative effect of the three mutations, we cotransfected the mutant and wild-type plasmids with ratio 1:1 into HEK293 cells. The γH2AX level in cotransfected cells after recovery for 3 or 6 hours were lower than that in cells isolated transfected with mutant plasmids. Three independent experiments were conducted (NC = negative control, which was transfected with pcDNA3.1 vector). (**B**) The relative grayscale of γH2AX after etoposide (ETO) treatment compared with no ETO treatment according to the Western blot image of three independent experiments (the grayscale of no ETO treatment was considered as 1 in each group of transfected cells). Independent sample *t*-test was used to compare the relative γH2AX. (**C**) Homologous recombination (HR) reporter system working principle. (**D**) The HR efficiency of wild-type or mutant MCM9 compared with NC group. (NC = cells transfected with I-SceI-vector and pcDNA3.1 vector.) Three independent experiments were conducted. *$P<.05$.

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SNPs rs768968338 and rs79670608 were substantially different when we compared our POI findings with the 1000 Genomes Project database, which also indicates the association between POI and *MCM9*.

Our DNA repair assays showed that the cells overexpressing mutant *MCM9* had impaired DNA-repair capacity, and we speculate about the haploinsufficiency of *MCM9*. Nearly all the biallelic variation carriers of *MCM9* presented with

primary amenorrhea. In sporadic POI, Desai et al. (22) found 1 in 151 patients carried homozygous variations of *MCM9*, while seven cases (7 of 151, 4.6%) had heterozygous variations with potential pathogenetic effects. The heterozygous carriers most likely experienced secondary amenorrhea, which would be consistent with the phenotype observed in our cohort. Therefore, we assume that the effect of *MCM9* mutations on ovarian function might be dosage dependent. Haploinsufficiency of *MCM9* caused by heterozygous variations predisposes women to secondary amenorrhea owing to residual functional MCM9, whereas biallelic variations might lead to more severe defects in ovarian development leading to primary amenorrhea. Desai et al. (22) also found heterozygous variations in *MCM8*, *BRCA1*, and *RAD54L* combined with *MCM9* variations coexisting in patients, which indicates that the cumulative effect of genetic defects affects the clinical severity of POI (33).

More importantly, the relationship between *MCM9* and tumors has still been elusive. The *Mcm9* knockout mice had a high risk of hepatocellular carcinoma and ovarian tumors (19). Goldberg et al. (34) reported a homozygous mutation carrier of *MCM9* had early colorectal carcinoma and POI. However, the three mutation carriers in our study had no history of tumors at the time of our investigation. Long-term follow-up observation for tumors, especially for hormone-sensitive tumors, should be recommended for patients with *MCM9* mutations.

CONCLUSION

We identified novel pathogenic mutations in *MCM9* in Chinese women with POI, which further expands the genotype spectrum of *MCM9* in POI.

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REFERENCE

1. De Vos M, Devroey P, Fauser BC. Primary ovarian insufficiency. Lancet 2010;376:911–21.
2. Webber L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod 2016;31:926–37.
3. Jiao X, Ke H, Qin Y, Chen ZJ. Molecular genetics of premature ovarian insufficiency. Trends Endocrinol Metab 2018;29:795–807.
4. Qin Y, Choi Y, Zhao H, Simpson JL, Chen ZJ, Rajkovic A. NOBOX homeobox mutation causes premature ovarian failure. Am J Hum Genet 2007;81:576–81.
5. Jiao X, Qin Y, Li G, Zhao S, You L, Ma J, et al. Novel NR5A1 missense mutation in premature ovarian failure: detection in han chinese indicates causation in different ethnic groups. Plos One 2013;8:e74759.
6. Tiotiu D, Alvaro Mercadal B, Imbert R, Verbist J, Demeestere I, De Leener A, et al. Variants of the BMP15 gene in a cohort of patients with premature ovarian failure. Hum Reprod 2010;25:1581–7.
7. Liu H, Guo T, Gong Z, Yu Y, Zhang Y, Zhao S, et al. Novel FSHR mutations in Han Chinese women with sporadic premature ovarian insufficiency. Mol Cell Endocrinol 2019;492:110446.
8. Huhtaniemi I, Hovatta O, La Marca A, Livera G, Monniaux D, Persani L, et al. Advances in the molecular pathophysiology, genetics, and treatment of primary ovarian insufficiency. Trends Endocrinol Metab 2018;29:400–19.
9. Venturella R, De Vivo V, Carlea A, D'Alessandro P, Saccone G, Arduino B, et al. The genetics of non-syndromic primary ovarian insufficiency: a systematic review. Int J Fertil Steril 2019;13:161–8.
10. Wang J, Zhang W, Jiang H, Wu BL. Mutations in HFM1 in recessive primary ovarian insufficiency. N Engl J Med 2014;370:972–4.
11. Caburet S, Arboleda VA, Llano E, Overbeek PA, Barbero JL, Oka K, et al. Mutant cohesin in premature ovarian failure. N Engl J Med 2014;370:943–9.
12. De Vries L, Behar DM, Smirin-Yosef P, Lagovsky I, Tzur S, Basel-Vanagaite L. Exome sequencing reveals SYCE1 mutation associated with autosomal recessive primary ovarian insufficiency. J Clin Endocrinol Metab 2014;99:E2129–32.
13. AlAsiri S, Basit S, Wood-Trageser MA, Yatsenko SA, Jeffries EP, Surti U, et al. Exome sequencing reveals MCM8 mutation underlies ovarian failure and chromosomal instability. J Clin Invest 2015;125:258–62.
14. Wood-Trageser MA, Gurbuz F, Yatsenko SA, Jeffries EP, Kotan LD, Surti U, et al. MCM9 mutations are associated with ovarian failure, short stature, and chromosomal instability. Am J Hum Genet 2014;95:754–62.
15. Qin Y, Zhang F, Chen ZJ. BRCA2 in ovarian development and function. N Engl J Med 2019;380:1086–7.
16. Guo T, Zhao S, Chen M, Li G, Jiao X, Wang Z, et al. Mutations in MSH5 in primary ovarian insufficiency. Hum Mol Genet 2017;26:1452–7.
17. Tucker EJ, Grover SR, Bachelot A, Touraine P, Sinclair AH. Premature ovarian insufficiency: new perspectives on genetic cause and phenotypic spectrum. Endocr Rev 2016;37:609–35.
18. Nishimura K, Ishiai M, Horikawa K, Fukagawa T, Takata M, Takisawa H, et al. Mcm8 and Mcm9 form a complex that functions in homologous recombination repair induced by DNA interstrand crosslinks. Mol Cell 2012;47:511–22.
19. Luo Y, Schimenti JC. MCM9 deficiency delays primordial germ cell proliferation independent of the ATM pathway. Genesis 2015;53:678–84.
20. Lutzmann M, Grey C, Traver S, Ganier O, Maya-Mendoza A, Ranisavljevic N, et al. MCM8- and MCM9-deficient mice reveal gametogenesis defects and genome instability due to impaired homologous recombination. Mol Cell 2012;47:523–34.
21. Fauchereau F, Shalev S, Chervinsky E, Beck-Fruchter R, Legois B, Fellous M, et al. A non-sense MCM9 mutation in a familial case of primary ovarian insufficiency. Clin Genet 2016;89:603–7.
22. Desai S, Wood-Trageser M, Matic J, Chipkin J, Jiang H, Bachelot A, et al. MCM8 and MCM9 nucleotide variants in women with primary ovarian insufficiency. J Clin Endocrinol Metab 2017;102:576–82.
23. Wang H, Shi LZ, Wong CC, Han X, Hwang PY, Truong LN, et al. The interaction of CtIP and Nbs1 connects CDK and ATM to regulate HR-mediated double-strand break repair. PLoS Genet 2013;9:e1003277.
24. Laven JSE, Visser JA, Uitterlinden AG, Vermeij WP, Hoeijmakers JHJ. Menopause: genome stability as new paradigm. Maturitas 2016;92:15–23.
25. Stringer JM, Winship A, Liew SH, Hutt K. The capacity of oocytes for DNA repair. Cell Mol Life Sci 2018;75:2777–92.
26. Winship AL, Stringer JM, Liew SH, Hutt KJ. The importance of DNA repair for maintaining oocyte quality in response to anti-cancer treatments, environmental toxins and maternal ageing. Hum Reprod Update 2018;24:119–34.
27. Carlosama C, Elzaiat M, Patino LC, Mateus HE, Veitia RA, Laissue P. A homozygous donor splice-site mutation in the meiotic gene MSH4 causes primary ovarian insufficiency. Hum Mol Genet 2017;26:3161–6.
28. Weinberg-Shukron A, Rachmiel M, Renbaum P, Gulsuner S, Walsh T, Lobel O, et al. Essential role of BRCA2 in ovarian development and function. N Engl J Med 2018;379:1042–9.
29. Caburet S, Todeschini AL, Petrillo C, Martini E, Farran ND, Legois B, et al. A truncating MEIOB mutation responsible for familial primary ovarian insufficiency abolishes its interaction with its partner SPATA22 and their recruitment to DNA double-strand breaks. EBioMedicine 2019;42:524–31.

30. Griffin WC, Trakselis MA. The MCM8/9 complex: A recent recruit to the roster of helicases involved in genome maintenance. DNA Repair (Amst) 2019;76:1–10.
31. Yatsenko SA, Rajkovic A. Reproductive aging and MCM8/9. Oncotarget 2015;6:15750–1.
32. Dou X, Guo T, Li G, Zhou L, Qin Y, Chen ZJ. Minichromosome maintenance complex component 8 mutations cause primary ovarian insufficiency. Fertil Steril 2016;106:1485–9.e2.
33. Yang X, Touraine P, Desai S, Humphreys G, Jiang H, Yatsenko A, et al. Gene variants identified by whole-exome sequencing in 33 French women with premature ovarian insufficiency. J Assist Reprod Genet 2019;36: 39–45.
34. Goldberg Y, Halpern N, Hubert A, Adler SN, Cohen S, Plesser-Duvdevani M, et al. Mutated MCM9 is associated with predisposition to hereditary mixed polyposis and colorectal cancer in addition to primary ovarian failure. Cancer Genet 2015;208:621–4.

Nuevas mutaciones patogénicas en el componente 9 del complejo de mantenimiento de minicromosomas (MCM9) responsable de la insuficiencia ovárica prematura

Objetivo: Investigar si las mutaciones en el gen del componente 9 del complejo de mantenimiento de minicromosomas (MCM9) estaban presentes en 192 pacientes con insuficiencia ovárica prematura esporádica (IOP) de ascendencia china.

Diseño: Estudio genético y funcional.

Ubicación: Centro universitario de medicina reproductiva.

Paciente(s): Un total de 192 pacientes con IOP esporádico y 192 mujeres control con menstruación regular.

Intervención(es): La secuenciación de Sanger se realizó en 192 pacientes con IOP esporádicos, y las posibles variantes patogénicas se excluyeron en los controles pareados. Los efectos funcionales de las mutaciones en MCM9 se exploraron en función de la respuesta al daño del ADN inducida por etopósido, y la capacidad de reparación del ADN se evaluó mediante el nivel de fosforilación de histona H2AX.

Principales medidas de resultado: Secuenciación de Sanger y características funcionales.

Resultado(s): Tres mutaciones heterocigotas novedosas en MCM9, c.C1423T (p.L475F), c.T2921C (p.L974S) y c.G3388A (p.A1130T), se identificaron en tres pacientes con POI por separado, que fueron ausente en 192 controles. Los estudios funcionales mostraron que las células del riñón embrionario humano 293 (HEK293) que sobre expresan el MCM9 mutante presentaban una capacidad de reparación de ADN disminuida en comparación con el tipo salvaje.

Conclusión(es): Este estudio identificó nuevas mutaciones en MCM9 que son potencialmente causantes de POI esporádicos en mujeres chinas y destacó aún más el papel de la capacidad de reparación del ADN en el mantenimiento de la función ovárica.